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A STUDY OF METHODS OF ESTIMATION OF METABOLIC NITROGEN

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INTRODUCTION

The so-called metabolic nitrogen of the feces is that portion which has an origin other than as an undigested food residue. It consists of residues from the bile and digestive juices, of epithelium and mucus from the digestive tract, and of such products of bacterial activity as have been derived from digested or from digestible nitrogen.

Our reason for wishing to estimate this fraction of the nitrogen of the feces is that it is a factor which must be considered in the determination of the digestibility of protein—a matter of great importance in relation to practical animal and human nutrition.

The plan of this experiment was to feed a basal ration of corn alone to each of five pigs during the first period, and to add to this corn ration in subsequent periods nitrogenous supplements to be used in the comparison of methods. In the selection of these supplements it was our object to choose foods the protein of which would probably be entirely digestible. Those used were milk, blood albumen, and commercial dried egg albumen.

In the comparison of methods of metabolic-nitrogen estimation it was our object to determine which procedure would yield results representing these assumedly entirely digestible protein foods as being entirely digestible—that is, assuming the proteins of milk, for instance, to be entirely digestible, we made an effort to determine which method of estimation of metabolic nitrogen would assign to the protein of milk a digestion coefficient nearest to 100 per cent.

An experimental study involving so much assumption can not yield results of the highest value, but it was our hope that it might assist in the establishment of a useful conventional procedure.

The methods of metabolic-nitrogen estimation compared in this study were the acid-pepsin method, the acid-pepsin and alkaline-pancreatin method, and the alcohol, ether, hot-water, and cold-lime-water method suggested in 1888 by Jordan.[1]

The philosophy of the two methods first mentioned is that by the use of digestive enzyms the nitrogen which has been digested, absorbed, and returned to the feces may be separated from the indigestible nitrogen. In using either of these methods we assume that there is no further digestion, during the course of the estimation, of that part of the food protein which escaped digestion in the alimentary tract of the experimental subject. We have no means of proving the truth of this assumption.

The acid-pepsin method represents stomach digestion alone. The acid-pepsin and alkaline-pancreatin method more nearly follows the physiological process, in that intestinal digestion is also represented. The latter method naturally yields decidedly higher results.

In the Jordan method the treatment with solvents is designed especially for the purpose of washing out bile residues, protein cleavage products and mucin.

The exact procedures followed in the three methods are as follows:

ACID-PEPSIN METHOD

Weigh out 5-gm. samples of fresh feces from a weighing bottle; roll up in 9 cm. filter papers, and transfer to 200-c. c. volumeteric flasks. Add 100 c. c. of pepsin-hydrochloric-acid solution (made by adding 1.25 gm. of pepsin to each liter of 0.33 per cent hydrochloric-acid solution). Shake thoroughly and put into an air bath maintained at 38° to 40° C. Allow the digestion to continue for 24 hours. During the first 6 hours agitate by rotation once each hour; agitate again 1 hour before final removal from the air bath. Arrange funnels with 12.5 cm. fluted quantitative papers, and dry 100-c. c. volumetric flasks. Promptly at 24 hours from the time of starting the digestion remove the 200-c. c. flasks from the oven, cool, fill to the mark with cold distilled water, mix thoroughly, and filter. Determine the nitrogen in 100 c. c. of the filtrate. The result represents metabolic nitrogen.

ACID-PEPSIN AND ALKALINE-PANCREATIN METHOD

Weigh 1.5 to 2.5 gm. samples of fresh feces into 150 c. c. Jena beakers. Add 100 c. c. of acid-pepsin solution (1.25 gm. of pepsin to each liter of 0.33 per cent hydrochloric acid). Stir thoroughly with a glass rod and place in an air bath maintained at 38° to 40° C. Stir thoroughly once each hour for the first 8 hours. Allow the digestion to continue for exactly 24 hours. Filter immediately through 12.5 cm. fluted quanti-

[1] [Jordan, W. H.] Analytical and experimental methods. Protein digestion. *In* Maine Agr. Exp. Sta. Ann. Rpt. 1888, p. 197. 1889.

tative filters. Wash beakers, filters, and contents until free from acid, with water at a temperature of 40° C.

Return filters and contents to the proper beakers and treat with 100 c. c. of alkaline-pancreatin solution (1.5 gm. pancreatin in somewhat less than 1 liter of water; add 3 gm. of sodium carbonate (Na_2CO_3); dilute to exactly 1 liter, and mix thoroughly). Return the beakers to the bath and stir thoroughly. Allow to digest for exactly 12 hours. Filter immediately through fluted papers. Wash beakers, filters, and contents thoroughly and repeatedly with hot water, and allow to dry. Transfer the filters and contents to Kjeldahl flasks and determine the nitrogen in the usual manner. Subtract the result from total nitrogen of the feces; the remainder represents metabolic nitrogen.

JORDAN'S METHOD[1]

Weigh 2 to 3 gm. portions of fresh feces, and dry at 100° to 105° C.; transfer to extraction capsules and extract with ether for 16 hours. Transfer to 150-c. c. beakers and treat with 50 c. c. of boiling 95 per cent alcohol. Keep at boiling temperature for 10 minutes; decant the alcoholic extract through qualitative filters; wash several times with hot alcohol and once or twice with ether, by decantation. With a camel's-hair brush transfer the residue from the filter papers to the original beakers; add 50 c. c. of hot water and boil for 10 minutes; filter through the same papers used for the last filtration, washing with hot water, by decantation. Wash the residues from the filter papers back into the beakers with 50 c. c. of a saturated solution of calcium hydrate, and let stand for 6 hours; filter through the same filters last used; transfer all the material from the beakers to the filter papers; wash with lime water, and allow to drain. Transfer filter papers and contents to Kjeldahl flasks, and determine the nitrogen. Subtract result from total nitrogen of the feces; the remainder represents metabolic nitrogen.

EXPERIMENTAL PROCEDURE

The subjects of this experiment were five Yorkshire barrows of nearly uniform age and weight. The average weight at the end of the first period was 53.85 kgm., and at the end of the fourth, 59 days later, 84.42 kgm., the average daily gain in weight being 518 gm., or 1.14 pounds. They were confined in the metabolism crates illustrated in our previous publications.[2]

[1] Jordan, W. H., Op. cit. (Detailed specifications were not submitted in the original publication; the particulars as here stated were arbitrarily assumed.)

[2] Forbes, E. B., Beegle, F. M., and others. A chemical study of the nutrition of swine. Ohio Agr. Exp. Sta. Bul. 271, p. 224–261, 3 pl. 1914.

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The experimental periods were of 10 days' duration, separated by 7-day intervals during which were fed the rations of the periods to follow. The feces were marked with carmine.

Table I records the total amounts and nitrogen content of the foods consumed and feces produced.

Table II records the percentages of total nitrogen in the feces and of metabolic nitrogen, as estimated by the three different methods.

Table III records the coefficients of digestibility of the nitrogen of the foods.

TABLE I.—*Foods consumed and total nitrogen in foods and feces*

Period No. (10 days).	Pig No.	Foods consumed.		Nitrogen in foods.		Weight of feces.	Total nitrogen of feces.	
		Corn.	Supplements.	Corn.	Supplements.			
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent.*	*Gm.*
I.....	1	18,200	.....	245.700	.....	6,357	0.995	63.252
	2	19,731	.....	266.373	.....	7,553	.889	67.146
	3	20,000	.....	270.000	.....	7,954	.989	78.665
	4	18,000	.....	243.000	.....	5,866	1.005	58.953
	5	16,800	.....	226.800	.....	5,645	1.000	56.450
			Blood albumen.		Blood albumen.			
II.....	1	16,200	730	215.298	83.987	5,737	.888	50.945
	2	18,000	810	239.220	93.191	6,144	.785	48.230
	3	19,000	855	252.510	98.368	6,710	1.006	67.503
	4	17,100	770	227.259	88.589	5,428	.892	48.418
	5	17,100	770	227.259	88.589	5,226	.943	49.281
			Skim milk.		Skim milk.			
III....	1	17,100	21,400	230.679	111.708	5,857	1.082	63.373
	2	19,000	23,800	256.310	124.236	6,566	.900	59.094
	3	19,000	23,800	256.310	124.236	6,936	1.059	73.452
	4	17,100	21,400	230.679	111.708	5,672	1.066	60.464
	5	17,100	21,400	230.679	111.708	5,529	1.060	58.607
			Egg albumen.		Egg albumen.			
IV.....	1	17,100	770	234.441	87.226	5,129	1.226	62.882
	2	19,000	855	260.490	96.854	5,847	.989	57.827
	3	19,000	855	260.490	96.854	6,263	1.346	84.300
	4	17,100	770	234.441	87.226	5,410	1.177	63.676
	5	17,100	770	234.441	87.226	5,371	1.070	57.470

TABLE II.—*Total and metabolic nitrogen of feces (per cent)*

Periods (10 days).	Pig No.	Total nitrogen.	Metabolic nitrogen.		
			Pepsin-hydrochloric acid method.	Pepsin-pancreatin method.	Jordan's method.
I	1	0.995	0.706	0.836	0.473
	2	.889	.619	.760	.418
	3	.989	.701	.834	.468
	4	1.005	.656	.816	.447
	5	1.000	.741	.858	.445
II	1	.888	.585	.749	.422
	2	.785	.581	.677	.404
	3	1.006	.773	.857	.446
	4	.892	.641	.738	.368
	5	.943	.712	.820	.380
III	1	1.082	.714	.831	.426
	2	.900	.590	.693	.347
	3	1.059	.739	.857	.392
	4	1.066	.704	.814	.416
	5	1.060	.691	.859	.420
IV	1	1.226	.802	.965	.525
	2	.989	.670	.761	.451
	3	1.346	.935	1.123	.599
	4	1.177	.729	.949	.565
	5	1.070	.742	.840	.433

TABLE III.—*Coefficients of digestibility of nitrogen*

Period No. (10 days).	Pig No.	Apparent digestibility.a	Pepsin-hydrochloric acid method.	Pepsin-pancreatin method.	Jordan's method.
I (corn)	1	74.26	92.52	95.89	86.49
	2	74.79	92.34	96.34	86.64
	3	70.86	91.52	95.43	84.65
	4	75.74	91.58	95.44	86.53
	5	75.11	93.55	96.47	86.19
II (blood albumen)	1	105.33	98.48	101.04	102.80
	2	112.96	106.22	102.27	109.18
	3	106.18	105.87	101.57	101.20
	4	107.58	106.22	102.26	102.45
	5	108.22	102.92	101.80	102.21
III (skim milk)	1	96.42	96.15	95.33	93.50
	2	104.44	99.42	96.61	98.34
	3	100.00	99.63	98.15	94.43
	4	95.97	99.01	96.62	94.81
	5	98.93	95.06	97.34	96.84
IV (egg albumen)	1	97.09	95.17	95.70	95.09
	2	108.10	101.34	96.08	103.45
	3	91.33	96.23	97.87	92.98
	4	92.20	94.84	98.12	98.25
	5	101.01	97.14	95.33	97.89

a On basis of total nitrogen of the feces.

CONCLUSIONS

The apparent digestibility of the protein of corn, based on the total nitrogen of the feces is about 75 per cent. On account of the existence in the feces of nitrogen of metabolic origin we know that the real digestibility is higher. The acid-pepsin method makes it appear that the real digestibility of the protein of corn is about 92 per cent, and the pepsin-pancreatin method about 96 per cent. Jordan's method gives appreciably lower figures, averaging 86 per cent.

The acid-pepsin method indicates that 70 per cent, the pepsin-pancreatin method 84 per cent, and the Jordan method 46 per cent of the nitrogen of the feces from corn is of metabolic origin.

All of the methods make the nitrogen of blood albumen appear more than completely digestible, even the apparent digestibility being over 100 per cent; thus, the feeding of blood albumen with corn seems to increase the digestibility of the corn protein to an extent more than sufficient to offset the incompleteness of digestibility of the protein of this supplement.

With skim milk the apparent digestibility varies from 95.97 to 104.44 per cent, the average being 99.15. With the acid-pepsin method three out of the five figures average 99.35. In previous work [1] five estimations by this method averaged 99.12. With the pepsin-pancreatin method the results were lower than with the acid-pepsin method. These low results on the supplementary food are reciprocals of the high results on the basal ration of corn.

The proteins of skim milk are made to appear more nearly completely digestible by the acid pepsin method than by the pepsin-pancreatin method or by the Jordan method.

With egg albumen the results varied considerably, but all were high. It would appear that raw, commercial, dried egg albumen is almost perfectly digested by swine.

Important inaccuracy seems to be inevitable in any determination of digestibility of supplementary foods in the usual way, by difference; and no other method seems more satisfactory. This applies equally to computations of real digestibility, and of apparent digestibility (based on total nitrogen of the feces).

The digestion coefficients for protein involved in the feeding standards of our reference works on animal production assume that the nitrogen of the feces is entirely an indigestible food residue. The rough measures afforded by the results of this study indicate that, as applying to the digestive capacities of swine, this assumption underestimates the digestibility of protein by about 20 per cent.

By way of interpretation of the individual variations in the digestion coefficients we would record the fact that pig 1, in Period II, manifested

[1] Forbes, E. B., Beegle, F. M., and others. Op. cit.

a pronounced dislike for the blood albumen. The acid-pepsin method indicates that this pig was less able than others to digest this foodstuff. Also, we would observe that, in response to most insistent demands for food, we gave to pig 5 in Period II a larger allowance of food per unit of body weight than was given to the other individuals. The digestion coefficient for blood albumen, as determined by the acid-pepsin method, with this pig also is low. Further, these two pigs, No. 1 and 5, had the lowest digestion coefficients, as determined by the acid-pepsin method, in the following period, No. III, where skim milk was fed.

In a study of the effects on metabolic nitrogen of storage of the feces in a frozen condition for 20 days, with and without the addition of thymol, compared with air-drying the fresh material, with and without thymol, no significant differences were observed which could be related to these methods of preservation.

In attempting to choose between these methods it seems to us that the acid-pepsin and the pepsin-pancreatin methods give results which are more nearly true than does Jordan's method, since the latter does not digest the bacteria, which may contain large proportions of the nitrogen of the feces and which presumably are more largely the product of digestible than of indigestible protein; but it is idle to attempt close comparisons of such conventional and inaccurate procedures. We have no accurate scientific basis for the determination of the digestibility of protein.

A NEW STRAIN OF RHIZOCTONIA SOLANI ON THE POTATO

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INTRODUCTION

Investigators heretofore have spoken of strains of *Rhizoctonia solani* Kühn when referring to cultures isolated from different hosts as well as to different isolations from the same host. Thus, Duggar[2] states (p. 423):

> The potato is the most interesting of the host plants with respect to the parasitism of *Rhizoctonia* by reason of the many types of disease induced under diverse conditions. The conditions may be in part climatic and, in part perhaps, dependent upon the pathogenicity of the particular strain of the fungus . . .

He further states (p. 442):

> Strains do occur, however, evidence of which may persist for some time in the general appearance of the cultures.

Edson[3] says—

> Much confusion exists regarding the identity of the various forms, and there is likewise great diversity of opinion as to the pathogenic properties of the members of the group.

Thus, while it is recognized that differences do exist, it has not been shown that it is possible to distinguish the different strains, especially from cultures obtained from the same host, either from their morphology or their growth on various media. Thus, Peltier[4] makes the following statement:

> . . . hence, on the measurement of the mycelial cells of *Rhizoctonia Solani*, as on the study of the growth on media, no conclusions can be based in regard to the distinguishing strains of this difficult species.

The purpose of this paper is to present evidence that two strains of *R. solani* are found on the potato (*Solanum tuberosum*), and, further, that it is possible to distinguish these with accuracy from the macroscopic growth on various media, as well as by the more accurate morphological comparisons.

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[1] The writers are indebted to Dr. H. A. Edson, of the Office of Cotton, Truck, and Forage-Crop Disease Investigations, for advice and suggestions during the progress of this work.

[2] Duggar, B. M. Rhizoctonia crocorum (Pers.) DC. and R. solani Kühn (Corticium vagum B. & C.), with notes on other species. *In* Ann. Mo. Bot. Gard., v. 2, no. 3, p. 403-458, 9 fig. 1915. Bibliography, p. 452-458.

[3] Edson, H. A. Seedling diseases of sugar beets and their relation to root-rot and crown-rot. *In* Jour. Agr. Research, v. 4, no. 2, p. 151. 1915.

[4] Peltier, G. L. Parasitic Rhizoctonias in America. Ill. Agr. Exp. Sta. Bul. 189, p. 372. 1916.

SOURCE OF MATERIAL

The cultures of *R. solani* on which the following studies are based were obtained from stems and tubers of potatoes grown in Florida and northern Maine. The isolations were made during the summers of 1915 and 1916, so that the comparisons are made with cultures of comparatively the same age. For the sake of convenience they are here designated "R1," "R2," "R3," etc. R1, R2, and R3 were isolated in Maine during the summer of 1916 from the base of affected plants. R4, R5, and R6 were obtained from the same locality from the inside of potato stems. R7 was isolated from tubers obtained at Hastings, Fla., in 1915, and R8 from tubers in Maine during the summer of 1915. Throughout these studies all the strains, from R1 to R8, could not be distinguished, with the single exception of R5. In presenting the results, therefore, R5 will be compared with a representative of one of the other cultures. The cultures from the stems were obtained from plants showing a girdling and hollowing at or near the surface of the ground (Pl. 25, *A*). This condition appeared to be a secondary stage in a malnutrition trouble briefly described by Edson and Schreiner.[1]

DISTINGUISHING CHARACTERS

The points of difference between R5 and the other strains, as here presented, can be grouped as pathological, as shown by inoculation experiments; physiological, as shown by the reactions on different media; and morphological, as shown by the measurements of mycelium, of surface sclerotial cells, and of diameters of germ tubes produced by germinating sclerotial cells.

PATHOLOGICAL CHARACTERS

Inoculations were made in the field on healthy growing plants. The method of procedure was to wash any dirt from the stems, make a slight incision with a flamed scalpel, and insert a bit of a young growing culture into the wound. The control plants were likewise injured. A number of such inoculations with R5, R1, R2, R3, etc., and a number of undetermined fungi also isolated from diseased stems, resulted in the production of very pronounced lesions with R5. The lesions produced by R1, R2, etc., were indefinite, as was also the case with all the undetermined fungi. The injured control plants remained healthy. Plate 25, *B*, illustrates the results of one of the series of inoculations. The three stems to the left were inoculated with R5, the next two with R1 and R2, and the last shows the condition of the control.

Inoculations were also repeated in the greenhouse, with results practically similar to those described above.

[1] Edson, H. A., and Schreiner, Oswald. A malnutrition disease of the Irish potato and its control. In Phytopathology, v. 7, no. 1, p. 70–71. 1917.

Inoculations on the tubers were also made. The method followed was to select clean and healthy tubers, immerse these for 10 minutes in a 1 to 1,000 mercuric-chlorid solution, wash with sterilized water, make a slight incision with a sterile scalpel, and insert a bit of a pure culture in the wound. Control tubers were treated in a similar manner, the agar medium without the fungus being inserted. The lesions produced with any of the strains were never very large, but a very distinct lesion was produced with R5 as compared with the other strain. Plate 26, *B*, illustrates a tuber inoculated with R5. For comparison a control tuber is shown in Plate 26, *C*. In several instances the lesion as a result of the inoculation extended to ¼ inch in diameter.

The strain R5, therefore, is not only more pathogenic on the stems than the remaining strains isolated from the potato, but is in fact able to produce a distinct necrosis of the tissues of the potato tuber.

PHYSIOLOGICAL CHARACTERS

The various strains were grown on a variety of media. Only those on which R5 has shown any marked distinguishing characteristics will be pointed out here.

POTATO AGAR.—On this medium, when grown in test tubes, R5 at the end of a week or 10 days produces a very marked discoloration of the medium. This coloration is dark brown, approaching black. The discoloration, if produced at all by the other strains on this medium, is very much less pronounced and never approaches the intensity of color produced by R5.

CORN-MEAL AGAR.—On this medium, when grown in test tubes, R5 produces light-gray, loosely formed sclerotia as compared with the darker, brownish, and more compact sclerotia formed by the other strains (Pl. 26, *A*). The character is very striking and can be relied upon.

USCHINSKY'S SOLUTION.—One hundred cubic centimeters of this solution was poured into 200-c. c. Erlenmeyer flasks and inoculated with small bits of pure cultures of the various strains grown on potato agar. The rate of growth of R5 is far in excess of the remaining strains. At the end of 10 days R5 entirely covered the surface and was growing on the side of the flask, while the growth of the remaining cultures were still below the surface of the liquid.

MORPHOLOGICAL CHARACTERS

In holding up against the light some petri-dish cultures on string-bean agar of the various strains, the writers were struck with an apparent difference in the fineness of the mycelial strands of R5 as compared with the remaining strains. A mount made and examined under the microscope revealed a distinct difference in the fineness of the mycelium. This difference in the diameter of R5 and the remaining strains was very evident

microscopically without definite measurements. Subsequent examinations made with the microscope, from cultures of various ages, as well as from a variety of different media, showed this difference constant, the diameter of the mycelium of R5 being in every case smaller than that of the remaining strains. Measurements of R5 and the remaining strains were made from cultures of the same age grown on the same media. The procedure followed was to grow the cultures in petri dishes, and mounts on slides were made a definite distance from the original planting. Fifty measurements were made at random in each case. The results of these measurements show that the diameter of R5 varies from 4.7 to 8.8 μ, with 7.8 μ as the average measurement, while the measurements of the remaining strains vary from 10 to 14.0 μ, with 10.1 μ as the average. Figure 1 illustrates this difference comparatively, both sections being drawn with the aid of the camera lucida, with the same magnification and from cultures of the same age. That the thickness of the mycelium is a good distinguishing character was confirmed as follows: 19 mounts were made from cultures grown on various media and of varying ages. The R5 was marked at the time of mounting, but in such a way that the person to whom they might be submitted was unaware of any distinguishing marks. These were submitted to two pathologists of the Office of Cotton, Truck, and Forage-Crop Disease Investigations, with a request that they divide the mounts into two lots, based on the diameter of the mycelium. In every case the mounts of R5 were separated from the remaining strains.

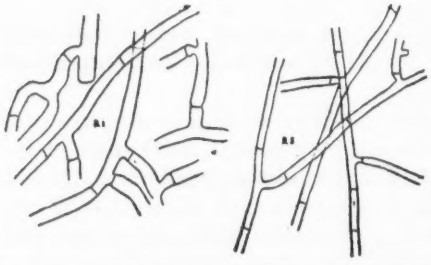

FIG. 1.—Camera-lucida drawings of mycelium of the R1 and R5 strains from the same medium and cultures of the same age.

Not only is the diameter of the mycelium of R5 smaller, but likewise the short sclerotial cells enveloping the sclerotia are smaller, as shown in figure 2. One hundred measurements show that those of R5 vary in length from 13.6 to 30.6 μ, with an average of 21.6 μ, while the others measure 17 to 61.2 μ, with an average of 37.5 μ. In width those of R5 measure from 8.3 to 20.4 μ, with an average of 12.3 μ. The other strains measure from 11.9 to 23.3 μ, with an average of 16.7 μ. Generally they are also much more regular than those found in the remaining strains, so much so, in fact, that they can be described as "monilia-like." In the case of the others, R1, R2, etc., while occasionally one sees a chain of regular sclerotial cells, this is the exception rather than the rule. It may perhaps be argued that R5 was contaminated with another sterile fungus the mycelium of which resembles that of a species of Rhizocotonia, but is of

a smaller diameter. It was not possible to obtain a culture from a single basidiospore, but dilution plates were made and a culture obtained from the germination of a single short typical sclerotial cell. This culture

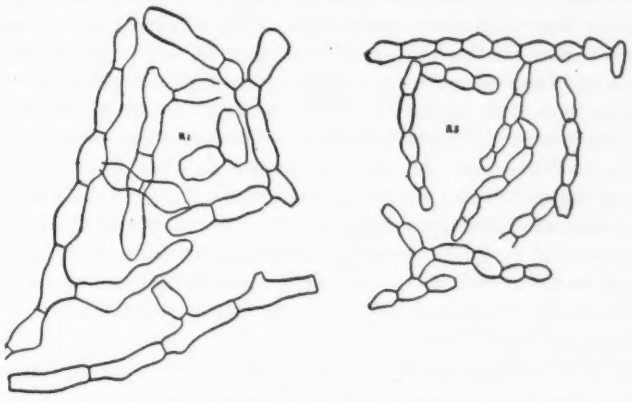

FIG. 2.—Camera-lucida drawings of short sclerotial cells of the R1 and R5 strains from cultures of the same age grown on the same medium.

agreed in all essential characters previously enumerated with the other cultures of R5.

While the mycelium measurements of R5 and all the remaining strains with which the writers worked are distinct, they do show variation, as indicated by the measurements given above. There may also be a question as to the accuracy of random measuring, even from mounts made from the same medium of the same age. A more accurate manner of identification of R5 from the remaining strains consists in the germination of the short sclerotial cells in water and the measurement of the diameter of the germ tubes produced. The mode of germination of the short sclerotial cells of R5 agreed in all essential characters with that of the remaining strains and has been described by Duggar[1] and others. Measurements made of the diameter of the germ tubes produced by R5 and the remaining strains

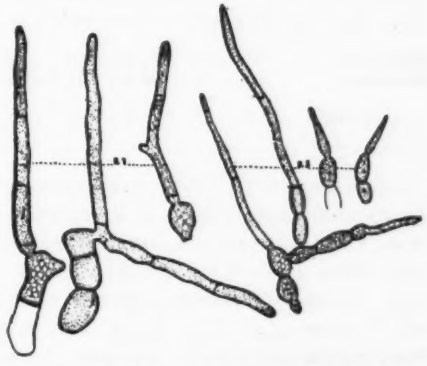

FIG. 3.—Camera-lucida drawings of germinating sclerotial cells of the R7 and R5 strains. The drawing was made at the end of 17 hours, after the cells were placed to germinate in drops of water.

[1] Duggar, B. M. Op. cit.

showed that the germ tubes produced by R5 are constantly smaller than those of the remaining strains. Figure 3 illustrates this relation. The writers consider this character the most reliable in the identification of these strains. In making the measurements of the germ tubes the question arose whether different length germ tubes produce tubes of varying width and also whether the size of the sclerotial cell is correlated with the size of the germ tube. In order to determine this point, 100 measurements were made of the short sclerotial cells, the length of the germ tube, together with the width of same. The results may be summarized as follows: The size of the cell has no apparent effect on the length or width of the germ tube; nor has the length of the germ tube any appreciable effect on the diameter of the germ tube. The width of the germ tubes produced by the germinating enveloping sclerotial cells of R5 vary from 3.4 to 6.8 μ, with an average of 4.3 μ, while those of R7 and R4, taken as representatives of the remaining strains, vary from 6.8 to 11.9 μ, with an average of 8.5 μ.

Table I summarizes the measurements made in the above comparative studies.

TABLE I.—*Comparative measurements of strains R5 and R7 of Rhizoctonia solani*

	Diameter of mycelium.		Sclerotial cells.				Diameter of germ tubes.	
			Length.		Width.			
	R5	R7	R5	R7	R5	R7	R5	R7
	μ	μ	μ	μ	μ	μ	μ	μ
Minimum	4.7	10.0	13.6	17.0	8.3	11.9	3.4	6.8
Maximum	8.8	14.0	30.6	61.2	20.4	23.3	6.8	11.9
Average	7.8	10.1	21.6	37.5	12.3	16.7	4.3	8.5

COMPARISON WITH RHIZOCTONIA CROCORUM (Pers.) DC.—Duggar gives the mycelial measurements of *R. crocorum* (Pers.) DC. as 4 to 8 μ; of *R. solani* Kühn, 8 to 12 μ. According to the measurements of the writers, R5 varies from 4.7 to 8.8 μ and the common strain of *R. solani* from 10 to 14 μ. These measurements agree so closely with those of *R. crocorum* and *R. solani* as given by Duggar that there appeared to be need for a closer comparison with *R. crocorum*. Such a comparison was made with exsiccatæ material, with material of *R. crocorum* on asparagus collected in Germany by Dr. H. A. Edson, of the Office of Cotton, Truck, and Forage-Crop Disease Investigations, and kindly furnished by him to the writers, and lastly with the descriptions and distinguishing characters as outlined by Duggar.[1] Unfortunately, the original specimens from which the isolations were made were not saved, as at that time it was not known that the strain of Rhizoctonia isolated was essentially different

[1] Duggar, B. M. Op. cit.

from the common strain of *R. solani*. It is therefore impossible to state with any degree of accuracy the color of the mycelium and sclerotia on the host, the presence or absence of infection cushions, etc. The writers, however, are reasonably sure that these characters were not those belonging to *R. crocorum*. Aside from the above consideration, its similarity to *R. crocorum* is shown by (1) the close agreement of mycelial measurements, (2) the sclerotia in culture approaching the plectenchymatic type. The differences are shown by (1) the myceliums lacking the typical violet or violet brown pigment in cultures of considerable age on a variety of media, (2) the branchings not being at right angles, though occasionally so, and (3) most important perhaps, the ease with which it is grown in culture on a variety of media contrary to the experience of all former investigators.

A consideration of the above facts lead the writers to believe that R5 is to be regarded as a distinct strain of the common *R. solani* Kühn as occurring on the potato rather than one of *R. crocorum* (Pers.) DC.

SUMMARY

A strain of *R. solani* Kühn, for the sake of convenience temporarily designated as "R5," was isolated from potato stems in Maine during the summer of 1916. This strain can be distinguished from the more common *R. solani* by (1) the more pronounced lesions produced when inoculated on injured stems or tubers; (2) the reaction, growth, and character of sclerotia on definite media; and (3) morphologically, by measurements of the mycelium, of the short sclerotial cells, and, lastly, by the measurement of the diameter of germ tubes when the short, or "barrel-shaped," cells enveloping the sclerotia are placed in drops of water to germinate.

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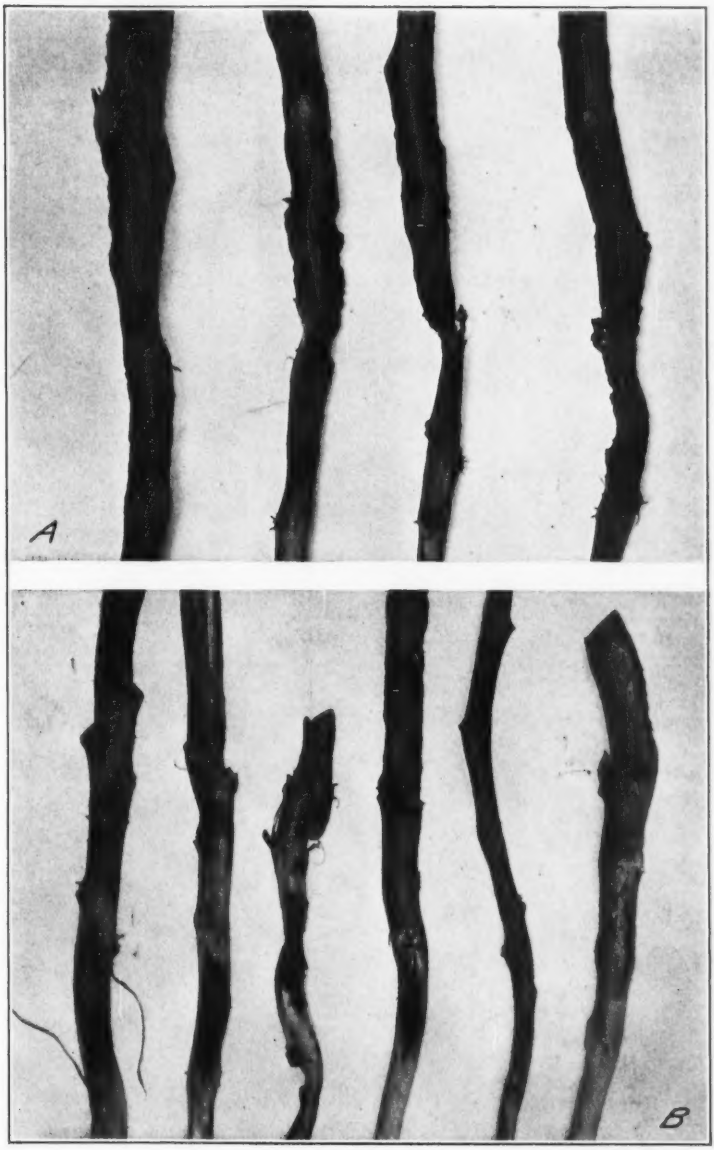
PLATE 25

A.—Potato stems, showing the nature of the lesions from which isolations of *Rhizoctonia solani* were made.

B.—Potato stems, showing the results of one series of inoculations. Three stems to the left were inoculated with strain R_5, the next two inoculated with R_1 and R_2, and the stem to the extreme right an injured control. The inoculations were made on August 14, 1916, and the photograph was taken on September 4, 1916.


A
B

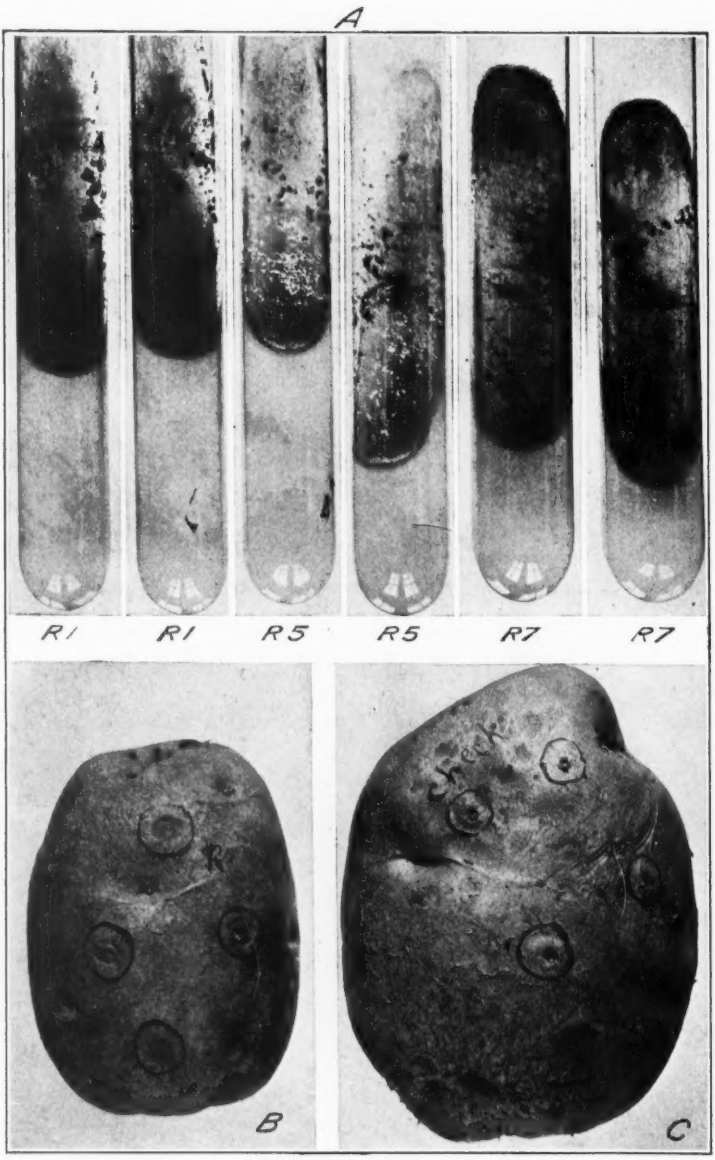

A
R1
R1
R5
R5
R7
R7
B
C

PLATE 26

A.—Reaction on corn-meal agar of R5, as compared with the other strains. Cultures were made on November 8, 1916, and were photographed on November 27, 1916. The two test tubes to the left show the character of the growth of strain R1, the next two those of R5, and the two test tubes to the right those of R7.

B.—A potato tuber, illustrating the results of inoculation with strain R5. The inoculation was made on November 5, 1916, and the photograph taken on November 15, 1916.

C.—An injured potato tuber (control). Photographed on November 15, 1916.

A FORM OF POTATO DISEASE PRODUCED BY RHIZOCTONIA

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What appeared to be an undescribed type of potato-tuber disease was observed a few years ago in southern Maine for the first time. Material from this region has been available for study during the present winter (1917). While there is no reason to think that it does not occur in other parts of the State, the writer has not seen any noticeable cases of this trouble in northern Maine, although all of the summer of 1916 was spent in the study of potato (*Solanum tuberosum*) diseases in Aroostook County.

This type of trouble was first recorded in Maine by Morse and Shapovalov.[2] Moreover, after examining a considerable number of potatoes received from various parts of the country as illustrating different types of potato-scab, they were led to make the following statement relative to this form of potato-tuber injury mentioned above:

> A critical examination of potatoes from a large number of sources, including those from other States, has convinced the writers that it is fairly common.

While several authors have mentioned scabbing, pitting, cracking, and ulcer formation more or less in association with Rhizoctonia, so far as the writer has been able to ascertain, with the exception of the publication mentioned above, no other references have been made in the literature to this type of scabbing or pitting, or, as the writer has chosen to call it, "dry core" of the potato tuber. Probably one of the nearest references to this type of trouble was made by Rolfs [3] in his description of an experiment with Rhizoctonia. He placed small amounts of hyphæ from pure cultures of the fungus on the surface of small growing potatoes and covered them with sterilized grafting wax. Fourteen days afterward two of these potatoes were examined, and a number of brown spots were found on the inoculated surfaces. Microscopic examination showed that the hyphæ had entered the lenticels and produced small ruptures in the skin. Further on he states that all of the inoculated tubers developed rough surfaces and cracks, but nothing was mentioned that would lead

[1] Thanks are due to Dr. W. J. Morse for materials and for valuable suggestions in formulating this paper.

[2] Morse, W. J., and Shapovalov, Michael. The Rhizoctonia disease of potatoes. Maine Agr. Exp. Sta. Bul. 230, p. 206. 1914.

[3] Rolfs, F. M. Potato failures. A preliminary report. Colo. Agr. Exp. Sta. Bul. 70, p. 11. 1902.

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June 18, 1917
Key No. Me.—10

one to believe that anything similar to our type of injury was developed. In a later publication[1] he makes this statement:

> European investigation long ago attributed the pitting or scabbing of tubers to the attacks of *Rhizoctonia*. Our experiments and observations also show that its attacks on growing tubers frequently produce deep ulcers. Most of our scab is due to the attacks of this fungus.

Other authors at about this same time observed Rhizoctonia hyphæ associated with the so-called scab ulcers, but were of the opinion that this fungus had nothing to do with their formation. However, in no case has the writer been able to find either photographs, drawings, or descriptions that give any adequate idea as to just what is meant by a scab ulcer.

Without a doubt this form of trouble has escaped the critical attention of pathologists for several years, owing to the fact that in some ways, upon superficial examination, it may frequently appear somewhat similar to the common scab of potatoes. However, upon careful comparison no one can have the slightest doubt but that this dry core or pitting is entirely different from the form of injury produced by *Oospora scabies* Thaxter, later considered by Lutman and Cunningham as identical with *Actinomyces chromogenus* Gasperini.

In greenhouse experiments Morse and Shapovalov[2] observed that this pitting was closely associated with Rhizoctonia. They found Rhizoctonia filaments within these pits and that infection apparently originated at the lenticels. However, their conclusions were based entirely upon general observations and upon the fact that the filaments of the fungus were constantly associated with all stages of the development of the diseased areas. No critical study was made of the relationship of the supposed parasite to the host tissues.

It is the purpose of this paper to describe this form of disease more fully and to present evidence which tends to show that *Rhizoctonia solani* Kühn (*Corticium vagum* B. and C.) is largely, if not entirely, responsible for this type of injury.

There are two phases of this disease that should be noted. First, the stage that on superficial examination might be mistaken for common scab. Second, a stage showing a canal formation which might be confused with the injury caused by the wireworm. The first stage is most generally noticeable where the infection is less than 3 mm. in diameter. However, there are exceptions to this.

The fungus enters at the lenticels and works its way down into the tuber without much external disturbance. The original outer cortex, being left more or less intact, forms a roof over the diseased area. The definite boundary and dark-brown color of the area suggest a form of scab. The interior granular mass of hyphæ, broken-down cells, and

[1] Rolfs, F. M. Potato failures. A second report. Colo. Agr. Exp. Sta. Bul. 91, p. 11. 1904.
[2] Morse, W. J., and Shapovalov, Michael. Loc. cit.

starch grains all remain in position, forming a dry "plug" and suggesting the name "dry core."

The second phase of this type of injury is most often found in the older stages in the progress of the disease or where the infected area reaches a diameter greater than 3 mm. This stage is most likely to be found when the potato tubers have reached maturity and the disease has run its course, or after the tubers have been stored. Owing to a drying out and shrinkage of tissues, a pit or canal is formed in the center of the affected area. Doubtless in harvesting and storing the tubers this pitting is furthered by parts of this granular material being shaken out or loosened. There is no doubt that the greater part of this pitting, particularly in cases where the disease has penetrated deeply into the flesh, has been attributed in Maine to the work of the wireworm. However, close observation even by the layman will readily show a great difference between these two types of injury.

The diseased areas are approximately circular in outline and at the surface vary in size from that of a lenticel to 6 or 7 mm. in diameter. They usually extend into the flesh of the tuber to a depth equal to or somewhat greater than the diameter. The dry core or pit thus formed gradually tapers off, forming a somewhat rounded end, very seldom becoming pointed. The majority of these pits are proportioned and shaped quite like a thimble, but some of them have a tendency to become longer and more slender. In such cases a dry, roughly cylindrical core may penetrate the flesh of the tuber for some distance. A casual observer might readily attribute this form of injury to insect attacks. In a few cases the pit has taken a more or less horizontal direction in reference to the surface of the tuber, and, as shown in Plate 27, *F*, two of these pits have joined together some little distance below the surface of the tuber.

Infection takes place in lenticels. Even from the very earliest stages the infected areas become slightly darker and sunken from the surrounding tissues. The mycelium of the fungus seems to travel equally in all directions; thus, as time passes, the infected area becomes a larger and larger circle dark brown in color. Surrounding the mature pit there is a very definite line of demarcation separating the diseased tissue from the healthy. In fact, this line is so definitely laid down that one has little or no difficulty in inserting the point of a knife and lifting out the whole core, leaving a clear-cut cavity in the healthy flesh of the tuber. By boiling tubers affected in this manner, the majority of the cores will come out clean with the peeling when it is removed. Plate 28, *E–I*, shows a group of these cores that came out with the peeling after the tuber has been boiled for 20 minutes. It will be seen (Pl. 29, *A*) that this division line is formed by three or four layers of compact suberized cells that have been laid down by the potato to prevent further penetration of its tissues by the fungal hyphæ. This leather-like lining of the

pit, once thoroughly established, effectively stops further progress of the fungus. No fungal filaments have ever been found penetrating this lining, and none have been found in the healthy tissues surrounding any of these pits. The evidence shows that the size of the pit is determined by the rate of suberization of cells sufficiently far enough in advance to effectively block further progress of the fungus. From very earliest infection, when the diseased area is scarcely more than an enlarged lenticel, the host cells always show more or less suberization three or four cells in advance of the deepest situated fungal filament. This being uniformly the case, together with the fact that the cells seem to die and lose their contents some distance ahead of the fungus, has suggested the possibility of a toxic substance being secreted by the hyphæ.

Microtome sections varying from 8 to 15 μ in thickness have been made from all stages of the "dry core" from the earliest, showing only the infected lenticel, to the age when the diseased area has ceased to enlarge, some as great as 6 mm. in diameter. In every case Rhizoctonia mycelium has been found. These infected areas are remarkably free from secondary fungi. In characteristic cases there is no histological evidence whatever of the presence of any other fungus. This, coupled with the fact that pure cultures of Rhizoctonia have been obtained repeatedly from these infections, seems to present a very strong case against *Rhizoctonia solani* Kühn (*Corticium vagum* B. and C.).

What appeared on the surface of a tuber as an enlarged lenticel is shown in section in Plate 30, *B*. After sectioning and staining this material, it was a matter of surprise to find Rhizoctonia hyphæ so readily, and in all sections made of this area. It will be seen from the illustrations that the host cells have already begun to suberize four cells in advance of the fungal hyphæ. No doubt this displacement and ragged edges of the normal corky cells of the cortex has been emphasized on account of their being washed and torn out in the process of fixing and cutting. However, it is believed that this will not prove misleading to anyone who has had experience in working with materials of this kind. The gradual progress of the fungus and the disintegration of the host cells, in what might be termed the second step, is shown in figure *C* (Pl. 30). This affected area was slightly over 1 mm. in diameter. By comparison with figure *A* (Pl. 30), which is a section of a normal portion of this same tuber, the results produced by the invading fungus may readily be seen. As the infection spreads and the fungal filaments become more and more abundant, this disintegration process goes on until the interior of the core is converted into a mass of broken-down cells, hyphæ, and free starch, thus giving an appearance, when magnified, similar to Plate 30, *D*. The hyphæ in this case will be seen to be of the sclerotia-forming type, as compared with the earlier infection stages or with the pure-culture hyphæ of figure *F* (Pl. 30). In a few cases the writer had the good fortune to get sections in which the outer cortex had remained more or less

intact, thus preventing the loss of the granular mass inside the core. Sclerotia-forming hyphæ were very abundant and in some instances, such as figured in Plate 30, *D*, cells more or less broken down were completely filled and apparently held in shape by the compact mass of mycelium. The mycelium of this character seemed to adhere to or follow the cell walls to a great extent.

The writer has seen little evidence of actual cell-wall penetration by the hyphæ. While Plate 29, *B*, seems to show that the fungal filaments can penetrate the walls, yet from the numerous sections made of diseased tissue practically no other cases could be found, although diligent search was made. The host cells are always found to have been killed, their walls suberized and more or less collapsed, quite some distance in advance of the fungal hyphæ. A falling apart and a separation of the cell walls suggest some kind of action upon the middle lamellæ. All of this action, taken together with the natural mechanical breakdown due to drying out and shrinkage, soon converts the interior of the core into a dry granular mass partially held together by the mycelium of the fungus.

Drayton,[1] in his study of Rhizoctonia lesions upon the potato stem, says:

> Individual hyphae were found running longitudinally and sometimes obliquely in the cells of these tissues and in the intercellular spaces.

This statement is made with reference to the vascular bundles and pith of the stem, and, although he does not actually say that cell-wall penetration takes place, yet one might infer that this does occur. It is believed, however, that this does not necessarily conflict with the present findings. Within the tuber the hyphæ find quite a different situation and seemingly have considerable difficulty in penetrating the cell walls.

SUMMARY

(1) Direct mention of this form of potato injury was first made in Maine. If reference elsewhere has been made to it, the descriptions were not adequate to warrant connection with this form of injury.

(2) Two phases of the injury are worthy of notice: One whose external appearance somewhat resembles scab and which extends as a dry core into the flesh of the tuber; another in which the shrinkage of tissues has formed a pit or canal in the center of the infected area, frequently suggesting wireworm injury.

(3) Careful histological studies of all stages in the progress of the injury invariably show the presence of Rhizoctonia hyphæ.

(4) Pure cultures of Rhizoctonia have repeatedly been obtained from the interior parts of the diseased areas.

(5) Evidence shows that the host cells die and lose their contents, and the walls suberize and are more or less broken down several cells in

[1] Drayton, F. L. The Rhizoctonia lesions on potato stems. *In* Phytopathology, v. 5, no. 1, p. 61. 1915.

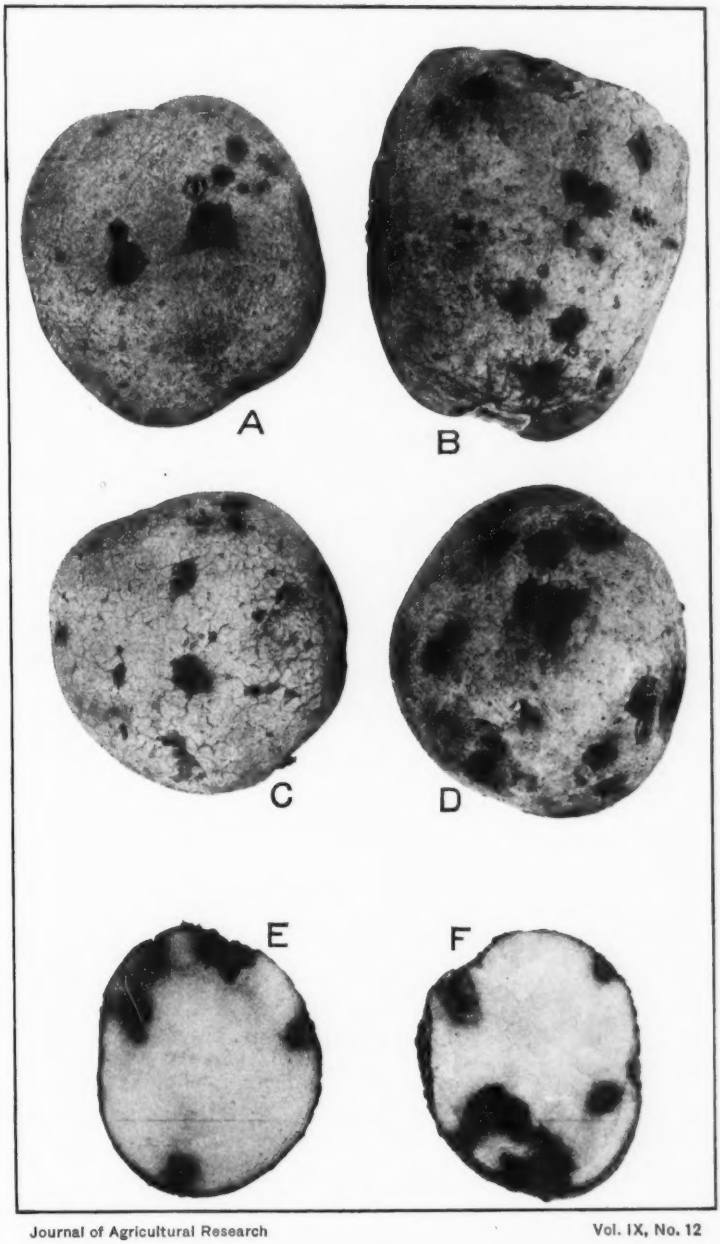
advance of the fungal filaments. This might lead one to suspect that part of the action is due to a toxin that is secreted by the fungus.

(6) Actual cell-wall penetration by the Rhizoctonia hyphæ apparently may occur, but this seems to be the exception rather than the rule.

PLATE 27

A–D.—Various stages of the "dry core" of potato tubers.

E–F.—Cross sections of a tuber badly affected with Rhizoctonia. Figure F shows two of the cores joined together. Compare with figure E of Plate 28, which illustrates a similar core taken bodily out of a boiled potato.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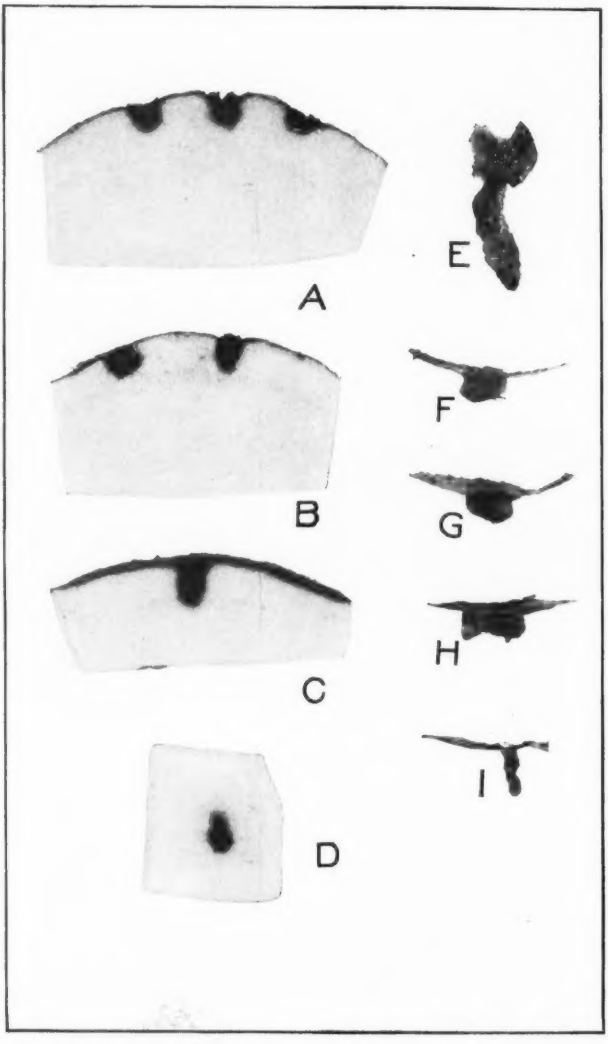
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PLATE 28

A–C.—A longitudinal section through some of the cores of affected potato tubers. Note the dry, granular mass still inside of the darkly outlined pits.

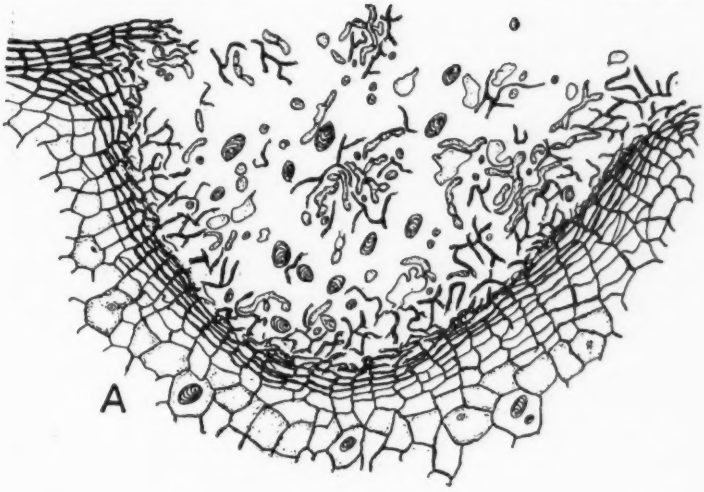
D.—Transverse section of a core.

E–I.—"Dry cores," giving a good idea of their size and shape. These were lifted out of a boiled potato.

PLATE 29

A.—A pit slightly over 2 mm. in diameter, showing broken down cells, free starch, and fungal hyphæ. Note the layer of suberized cells arising from the outer cortex and forming a lining to the pit.

B.—Fungal hyphæ apparently penetrating the cell walls.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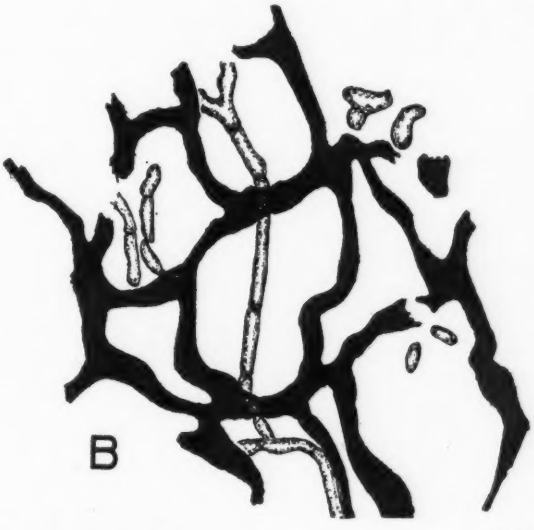
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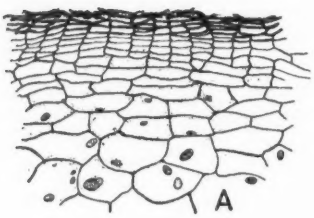
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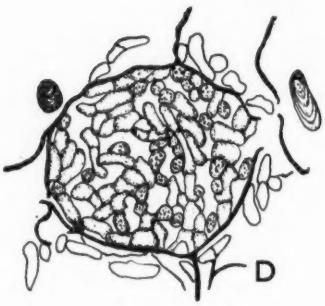
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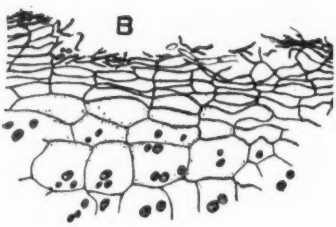
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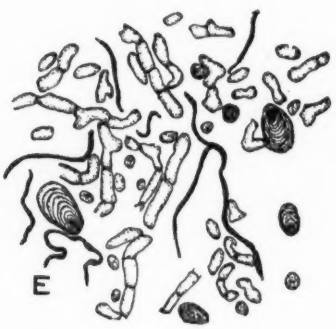
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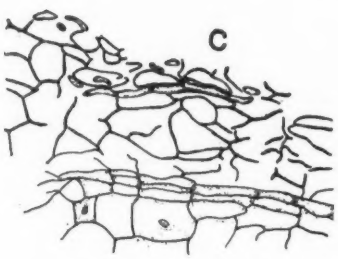
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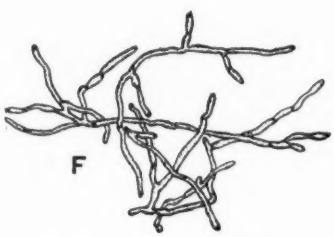
F

PLATE 30

A.—The normal cortex and inner tissues of a potato tuber.

B.—An enlarged lenticel, showing a very early stage of infection.

C.—Fungal filaments and broken down cells in an infected lenticel slightly over 1 mm. in diameter.

D.—A host cell highly magnified showing the interior filled with fungal hyphæ.

E.—The granular contents of one of the pits as it appears under the microscope.

F.—Rhizoctonia hyphæ from a pure culture isolated from the interior part of a dry core.

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